Pigment Fluorescence Signatures as an Index to the Taxonomic Structure of Phytoplankton Communities

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LONG-TERM GOALS

The spatial distribution and taxonomic composition of phytoplankton communities are important determinants of the structure of marine ecosystems and the optical environment of the sea. In oceanic waters the dominant photoautotrophs are picoplankton cyanobacteria. Dominant photoautotrophs in coastal waters, in contrast, are frequently larger diatoms, dinoflagellates and prymnesiophytes. The optical environment of Case 2 waters is shaped by the absorption and scattering of the larger phytoplankton with pigment signatures and cell, or colonial, morphologies that are distinct from the oceanic picoplankton in Case 1 waters (Kirk, 1994).

Biological oceanographers have developed a variety of methods to map the spatial distribution of taxonomic groups in natural plankton communities. The 'classical' approach of microscopic enumeration of preserved samples is now supplemented by molecular biological and molecular phylogenetic techniques adapted from medical research (Long, 1998). Furthermore, the distinct pigment composition of individual phytoplankton taxonomic classes can be used as pigment 'biomarkers' to qualitatively map the abundance of phytoplankton classes with high performance liquid chromatography. Fluorescence characteristics of phytoplankton classes have also been utilized in both microscopic and flow cytometric methods to identify taxa in natural communities (Wood *et al*., 1985). These methods are, however, mainly applied to discrete samples collected at specific locations and depths. Since the optical, physical, chemical and biological properties in the ocean vary over a wide of temporal and spatial scales (Bidigare *et al*., 1992), *in situ* observations are needed at frequent intervals in time and space to determine relationships between the abiotic and biological processes.

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This project began in spring, 2001 to develop a multiple wavelength fluorometer to map the fluorescence distribution of two taxonomic groups of phytoplankton, chromophytes and cyanobacteria, at length scales of 10s meters to kilometers. These length scales correspond to the scale variability in phytoplankton spatial distributions in coastal waters. The primary test region for our instrument is Florida Bay, a shallow estuary (see Fig. 1). Cyanobacterial blooms frequently occur in the north central Bay while larger diatoms are dominant in late summer to winter in western basins.

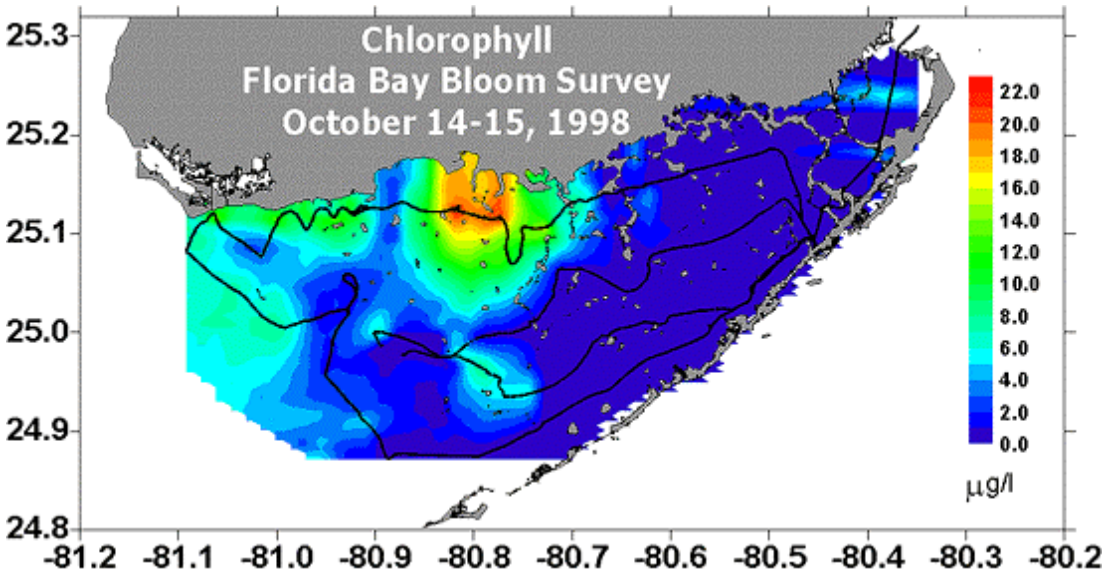

Figure 1. An example of the spatial distribution of chlorophyll a in Florida Bay from October 14-15, 1998. Peak concentrations in north central Bay > 20 µg l^{-1} (at 25.1°N, 80.8°W) correspond to a Synechococcus bloom. The localized maximum (ca. 10 µg l^{-1}) in the northwest Bay centered near 25.1°N, 81.0°W corresponds to a diatom bloom.

OBJECTIVES

1. Configure the optics and construct an autonomous, low-power multiple-channel fluorometer.

2. Evaluate the capability of matrices of fluorescence signatures to serve as indices to chromophytes and cyanobacteria. The validity of fluorescence signatures will be assessed from RP-HPLC analyses provided by colleagues with concurrent research programs in Florida Bay.

3. Map the temporal and spatial pattern of fluorescence signatures in a subtropical lagoon, Florida Bay.

APPROACH

Yentsch and Yenstch (1979) first proposed that fluorescence excitation and emission characteristics (fluorescence 'signatures') could serve as indices to phytoplankton groups. They hypothesized that the spectral fluorescence response could potentially discriminate among taxonomic groups such as diatoms, dinoflagellates and coccolithophrids. Subsequent studies have identified four categories of discernable fluorescence signatures, specifically those for chromophytes ('golden-brown' algae),

chlorophytes ('green' algae), cryptophytes-rhodophyceae, and cyanobacteria ('blue-green' algae). Carotenoids in chromophytes transfer energy to chlorophyll *a*, and although they do not fluoresce, the resulting chl *a* fluorescescence can be used as an index to the presence of chromophyte accessory pigments. Indices such as the chlorophyll accessory pigment (CAP) ratio have been proposed as a means to distinguish chromophytes, chlorophytes and cyanobacteria in marine waters based on the fluorescent response $F_{530:685}/F_{450:685}$ [where $F_{ex:em}$ corresponds to the fluorescence response at paired excitation (ex) and emission (em) wavelengths, respectively]. Subsequently, Watras and Baker (1988) used the ratio ($F_{630:660}/F_{430:680}$) to successfully distinguish freshwater cyanobacteria from other taxonomic groups, while Seppälä and Balode (1998) mapped cyanobacterial distributions by fluorescence signatures in the Baltic Sea. Cowles *et al*. (1993) have also utilized fluorescence signatures to describe the distribution of dominant taxonomic groups in marine waters.

In summer 2001 we began construction of the dual wavelength fluorometer. Excitation at two wavelengths, (F_{ex}) 480 nm and 520 nm, is each followed by measurements of emission intensity at (F_{em}) 570 nm and 680 nm. Excitation flashes are separated by 100 μseconds and the emission measurements are offset from excitation by 20 – 30 μseconds. This 4 component matrix can be used to readily distinguish between cyanobacteria and chromophytes. We recognize that a large source of variability in the fluorescence signatures of natural phytoplankton populations may result from physiological responses to changing environmental conditions. Factors such as light, nutrient status and cellular optical properties influence fluorescence responses (e.g., Soo Hoo *et al*., 1986). Thus fluorescence signatures should be considered a qualitative indicator of taxonomic composition.

WORK COMPLETED

A dual-wavelength fluorometer is nearing completion for laboratory tests of the system. Our initial tests in the laboratory include diatom, dinoflagellate, and cyanobacteria (*Synechococcus* spp. and *Prochlorococcus marinus*) cultures. The prototype electronics were constructed by BathySystems, Inc. with the optics based on an autonomous fluorometer developed for Lagrangian platforms (Hitchcock et al., 2000). The system is controlled by a Motorola microcontroller with separate triggers for the excitation light sources that presently consist of EG&G strobe lamps with appropriate interference filters.

RESULTS

We have measured the spectral responses of the taxonomic groups (chromophytes, chlorophytes and cyanophyceae) that can potentially dominate in Florida Bay. The fluorescence spectra were determined with laboratory cultures on a Hitachi F4500 fluorescence spectrofluorometer. Our results confirm Poryvkina *et al*. (1994), who described spectral signatures of 28 phytoplankton species as 2-dimensional excitation-emission matrices.

The field phase of this effort will be initiated in winter 2001 after laboratory tests are complete. Maps of fluorescence distributions will be derived from bimonthly surveys conducted by NOAA's South Florida Ecosystem Restoration Prediction and Modeling (SFERPM) program (for a description, see http://www.aoml.noaa.gov/ocd/sferpm/overview_text.html). Ancillary physical (temperature, salinity, position) and bio-optical (chlorophyll *a* and CDOM fluorescence, beam transmittance) data are

collected during the survey, and will be analyzed to determine spatial relationships of plankton distributions to physical and biological parameters.

IMPACT/APPLICATIONS

The principal test region for the dual wavelength fluorometer is Florida Bay, a subtropical lagoon with distinct regions characterized by seasonal blooms of cyanobacteria and diatoms. Red tides have also occurred in the Bay, although they are not an annual event. This estuary is the focus of a large interagency-funded research effort that evaluates options for various restoration plans of the Everglades (for details on the relationship of the Everglades restoration to Florida Bay, see documents listed at http://www.aoml.noaa.gov/flbay/index.html). We will incorporate the multi-channel fluorometer into the datastream from the SFERPM Florida Bay surveys to construct spatial maps of taxonomic groups in the Bay. 'Ground truth' measurements will be derived from HPLC analyses for pigments by Dr. W. Louda, FAU (Louda *et al*., 1998). Knowledge of the diatom (chromophyte) and cyanobacterial distributions, and their scales of variability, will provide insights as to how physical and biological forcings influence phytoplankton dynamics in the Bay.

TRANSITIONS

This project began in Spring 2001 and there are no current transitions.

RELATED PROJECTS

There are three related projects now studying the structure of plankton communities in Florida Bay. G. Hitchcock and a co-investigator (Dr. G Vargo, Univ. South Florida) are studying the role of phytoplanton and benthic microralgal communities in relation to nutrient cycling. Dr. M. Dagg (LUMCON) and Mr. S. Cummings (NOAA/AOML) are concurrently studying the impact of wind events on the resuspension of bottom sediments and microalgal communities as they influence food web structure in the surface waters. Dr. W. Louda of Florida Atalntic University is describing the structure of phytoplankton and benthic microalgal communities through high perfomance liquid chromatographic techniques, with emphasis on the fate of plant pigments in the water column and benthos. The central goal of these studies is to determine the role of pelagic and benthic microalgae in the structure and functioning of food webs in Florida Bay.

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